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Investigation of the Mode of Action of Nematode Neuropeptides*

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Abstract: The neuropeptide AF2 has a complex set of actions on the dorsal muscle strip of *Ascaris suum*, including a potentiation of the acetylcholine-stimulated muscle contraction. Caffeine at 100 μM and 5 mM inhibited this potentiation, as did 100 μM theophylline in two out of six studies. The cyclic-AMP-potentiating compounds IBMX, dibutyryl cAMP and forskolin had no effect on the AF2-induced potentiation of the acetylcholine-stimulated muscle contraction. These preliminary data suggest that the potentiating action of AF2 is not mediated by a cAMP pathway.

Key words: *Ascaris suum*, neuropeptides, nematode, caffeine.

1 INTRODUCTION

Studies on the nervous system of nematodes have shown that FMRFamide-related peptides (FaRPs) are widely distributed. Cowden *et al.* isolated two FaRPs from the parasitic nematode *Ascaris suum* Goeze, AF1 (KNEFIRFamide)[1] and AF2 (KHEYLRFamide)[2] and have recently isolated at least another 11 FaRPs from this organism. AF2 has also been isolated from the parasitic nematode *Haemonchus contortus* Rudolphi,[3] which suggests that this peptide may be conserved throughout the nematode phylum.

Five FaRPs have been isolated from the free-living nematode *Panagrellus redivivus* (L.) Goodey, PF1 (SDPNFLRFamide), PF2 (SADPNFLRFamide),[4] AF2, PF3 (KSAYMRFamide) and PF4 (KPNFIRFamide).[5–7] A precursor gene encoding a family of FaRPs (flp-1) has also been cloned from *Caenorhabditis elegans* Maupas.[8,9] From the precursor gene it can be deduced that the gene sequence for both PF1 and PF2 is present in *C. elegans*.

These peptides are bioactive both in whole worms, and in isolated dorsal muscle preparations from *A. suum*. The physiological importance of the AF-like peptides was first shown by Cowden and Stretton, who injected AF2 (1 nmol) into the anterior region of *A. suum* and noted that it caused a paralysis.[2] Furthermore, AF2 has complex actions on an isolated dorsal muscle strip preparation from *A. suum*.[2,10] The PF-like peptides have an inhibitory action on *A. suum* muscle.[7,11–13]

The cellular site of action of these peptides has not yet been determined. This paper sets out some preliminary experiments to address this problem.

2 MATERIALS AND METHODS

A. suum were obtained from the local abattoir and maintained for up to five days in artificial perienteric fluid (APF; in mM, sodium chloride 67, sodium acetate 67, calcium chloride 3, magnesium chloride 15·7, potassium chloride 3, Tris buffer 5 mM, pH 7·6 with glacial acetic acid) with glucose (3 mM) at 37°C. The dorsal

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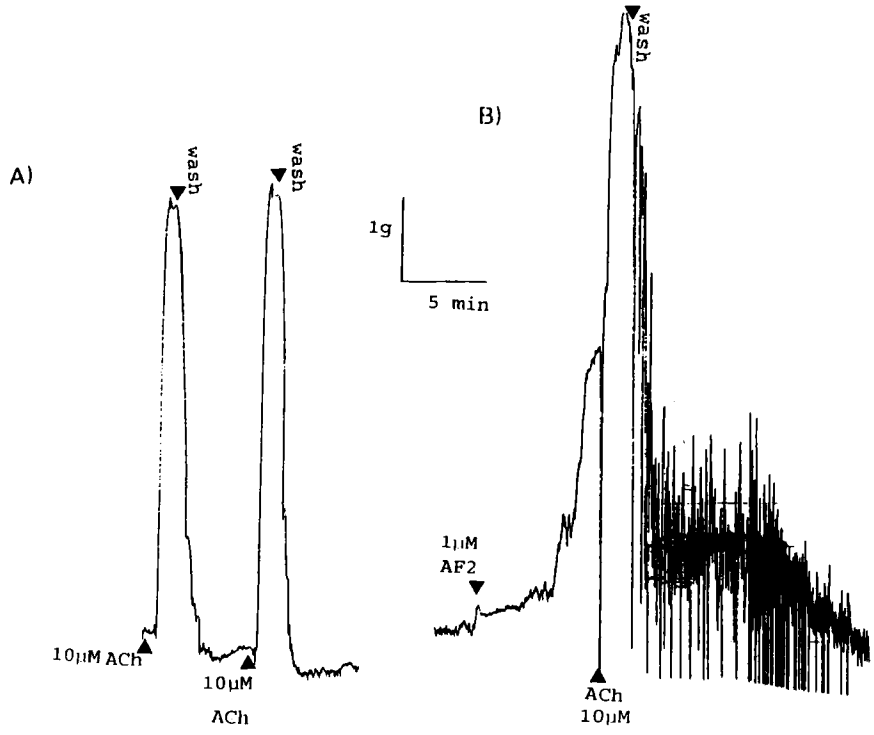

Fig. 1. *A. suum* muscle tension recordings from isolated *in-vitro* dorsal muscle preparations. In each recording, arrows indicate the addition of drugs. Both muscle strips are from the same worm. (A) Addition of 10 μM ACh to the dorsal muscle preparation. (B) Effect of 1 μM AF2 on the dorsal muscle, and the ACh-induced contraction.

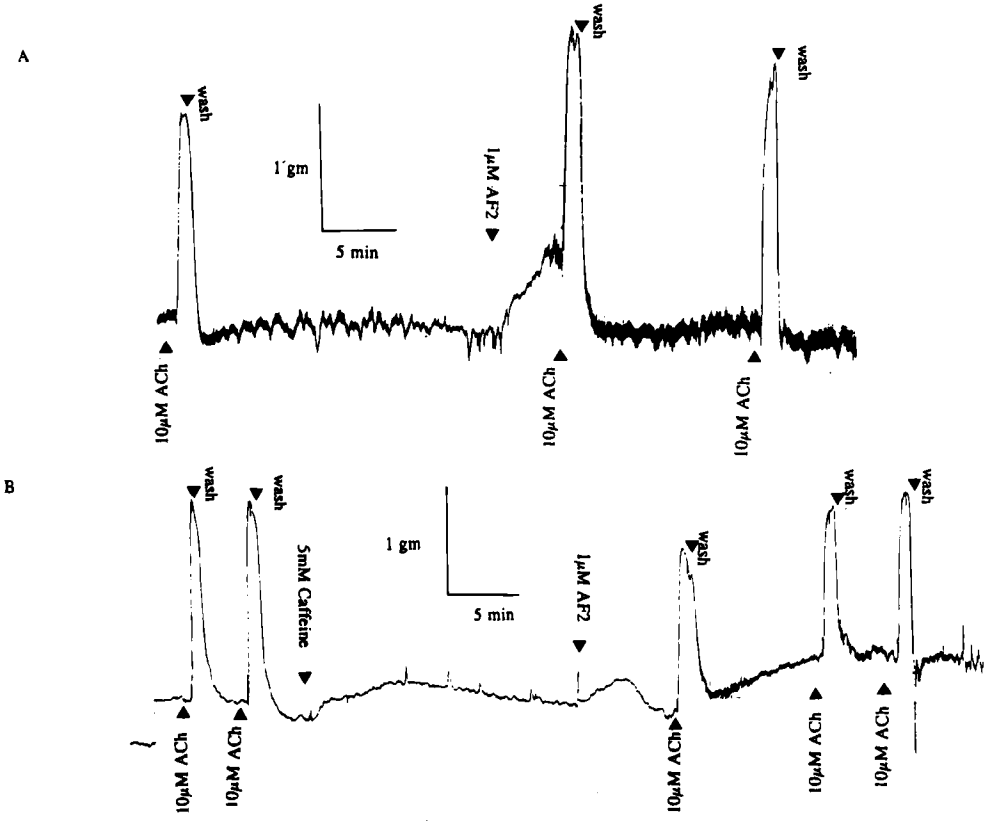

Fig. 2. Effect of 5 mM caffeine on the AF2-induced potentiation of the ACh response in *A. suum* dorsal muscle. (A): Control, (B): 5 mM caffeine preincubated for 20 min prior to the addition of AF2.

muscle strip preparation was made using methods described previously.[10]

2.1 Organ bath experiments

Effects on the resting tension and phasic activity were determined by securing a dorsal muscle strip in an organ bath (volume 10 ml) and attaching it to an isometric transducer at a resting tension of 2 g in APF at 37°C. Test compounds (100 μl volume) were added to the bath and the tissue was gassed with room air for 2–3 s to ensure fast and complete dispersion of the aliquot in the volume of the bath. A hard copy of the recording was obtained on a flat-bed chart recorder.

3 RESULTS

3.1 Organ bath experiments

Addition of 10 μM acetylcholine to the organ bath (Fig. 1(a)) produced a dose-dependent contraction of the muscle that was identical to that observed in previous experiments.[10] Addition of AF2 to the *A. suum* dorsal muscle strip elicited a dose-dependent contraction of the muscle (Fig. 1(b)), as has been previously described.[2,10] At 1 μM, AF2 potentiated the contraction elicited by acetylcholine (10 μM) by *c.*43% (Fig. 1(b)). AF2 (1 μM) gave rise to increased spontaneous activity of the dorsal muscle which was long-lasting (1–2 h to wash out) (Fig. 1(b)). In some, but not all, preparations, AF2 (0·5–1·0 μM) also elicited a biphasic response whereby a relaxation was observed prior to the muscle contraction (data not shown). Experiments were performed using a range of signal transduction modulators in an attempt to determine if any of the actions of AF2 on the dorsal muscle strip were mediated by second messenger pathways. One effect of AF2 on *A. suum* dorsal muscle is the potentiation of the acetylcholine (ACh) response. This effect is both reproducible and quantifiable. Compounds were added 20–30 min prior to addition of AF2. They were prepared in either dimethylsulfoxide (DMSO) or APF. The concentration of DMSO was kept at 1 ml litre^{-1} in the organ bath. Dibutyryl cAMP and 3-isobutyl-1-methylxanthine (IBMX) at 100 μM and Forskolin at 10 μM had no effect on the AF2-induced potentiation of contraction by ACh, nor did they appear to have any significant effect on the muscle itself. Theophylline caused a relaxation of the somatic muscle in three out of six experiments and, at 100 μM, in two out of six experiments, inhibited the AF2-induced potentiation of contraction by ACh by around 50%. Caffeine at 5 mM also produced an inhibition of the AF2-induced potentiation of contraction by ACh, (compare Figs 2(a) and (b)). Caffeine (5 mM) also gave rise to a small, long-lasting increase in basal tone of the somatic muscle. This effect was also seen at a caffeine concentration of 100 μM and the inhibition was reversible after a 5-min wash (data not shown).

4 DISCUSSION

As the results show, AF2 has a complex set of actions on the dorsal muscle preparation of *A. suum*, including a decrease and increase in basal tension and a long-lasting increase in the frequency and amplitude of spontaneous muscle contraction. These actions are probably mediated by more than one receptor, and probably involve both pre- and post-synaptic events.[14] Several of our experiments showed that AF2 had a biphasic response which has been previously described by Cowden and Stretton.[2] We have recently begun to investigate the site of action of nematode FaRPs, in order to understand the cellular pathway by which these peptides exert some of their effects. Although some of the actions of AF2 are probably due to a direct action on the somatic muscle, the long-lasting effect of this peptide, in addition to its role in potentiating the response to ACh, suggests that a second messenger system is involved. We have carried out preliminary experiments using the cAMP-potentiating compounds IBMX, dibutyryl cAMP and forskolin. None of these compounds had any significant effect on the muscle tissue alone, nor did they appear to effect the AF2-induced potentiation of the acetylcholine-stimulated muscle contraction. This suggests that AF2 does not exert its actions through a cAMP-mediated pathway.

Theophylline caused a relaxation of the somatic muscle in three out of six experiments. However in two out of six studies, 100 μM theophylline inhibited the AF2-induced potentiation of the ACh response by approximately 50%. Caffeine produced a similar inhibition at both 100 μM and 5 mM. Some neuropeptides are known to exert their actions through second messenger systems[15,16] and we are currently evaluating several additional compounds in an attempt to determine whether a second messenger system is involved in the AF2-induced potentiation of the Ach response in *A. suum* muscle.

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